REMARKS

1. Restriction Requirement (OA pp. 2-5)

1.1. The Examiner has maintained the previously raised restriction requirement following the Applicant's election with traverse. The Examiner argues that the common technical features of the disputed claims is anticipated by Bianchi et al and by Valverde et al.

We submit that the basis for the holding of a posteriori lack of unity is clearly untenable.

Since claims 14-16 incorporate all the features of claim 1, it is immediately apparent that all of the features of claim 1 are technical features that the claims have in common, and if claim 1 has an inventive step, then inevitably the claims relate to the same invention under PCT rules. See PCT Administrative Instructions, Annex B, paragraph (c). The Examiner recites that claim 1 requires both of:

1) an operative first metabolic pathway in which a first metabolite is transformed into a second metabolite in a reaction in which NAD is a cofactor for a first enzyme (producing NADH) and in which said second metabolite is transformed into a further metabolite by a second enzyme; and
2) a second metabolic pathway characterized by an enzyme activity in respect of a third enzyme in excess of the native level catalyzing a non-reversible reaction in which NADP is a cofactor producing NADPH.

The Examiner explains where in Bianchi et al the first feature is found, leading to production of 3-phosphoglycerate as the 'further metabolite' as we understand it.

The Examiner does not state where in Bianchi et al the second feature is to be found.

Instead, the Examiner remarks that it is unclear how the second metabolic pathway relates to the functional activity of

the third enzyme and whether the first metabolite is simultaneously transformed into a second and third metabolite or they are separate metabolic conversions.

Whether those matters really are unclear or not (and we say not - see below), this is clearly dodging the issue of whether in fact the second requirement numbered above is actually disclosed in Bianchi et al. Accordingly, there is no justification for the stated conclusion that the combined features are anticipated by Bianchi et al.

Secondly, the Examiner newly asserts that the features in question are anticipated by Valverde et al. The Examiner explains that Valverde et al teaches expression of (we abbreviate) GAPN in *E. coli* which would fulfil the role of the second metabolic pathway with the increased activity of the third enzyme.

However, in relation to Valverde et al, the Examiner does not explain where this teaches the first feature. Since in Valverde et al, GAPDH was deleted this is an insuperable difficulty. Thus, the engineered *E. coli* of Valverde et al lacks the required operative first pathway by virtue of the deletion of the NAD-dependent GAPDH.

The Examiner has not stated an argument that the common technical features of the claims lack inventive step based on a combination of Bianchi et al and Valverde et al. Perhaps it was intended. However, that would seem to be unlikely since no obviousness rejection combining these references has been made. Neither is it apparent how these two documents could be combined in an obvious way to produce anything having the required combination of features.

For the reasons stated below in relation to 35 USC §103, no valid objection that the claims relate to separate inventions (under the PCT) could be raised on the combination of Valverde et al and Nissen et al either.

We submit that claims 14-16 should be rejoined.

1.2. We note that the Examiner fails to reject the claims

as anticipated by either Bianchi or Valverde. Bianchi is not relied on at all, and Valverde is relied on only in combination with Nissen (see OA pp. 9-12). The Examiner should have either made these rejections or withdrawn the holding of a posteriori lack of unity based on anticipation by Bianchi or Valverde.

1.3. The Examiner states that PCT rule 13.2 no longer specifies the combinations of categories of invention which are considered to have unity of invention. This is ingenuous. That listing of safe combinations was simply moved to the PCT Administrative Instructions, Annex B, paragraph (e).

MPEP 1850(I), last two paragraphs, directs examiners to continue to consider the category combinations even though the categories are no longer explicitly recited in PCT rule 13.2. See also MPEP 1850 (III)(A).

Moreover, the combinations are expressly recited in 37 CFR 1.475(b)(1)-(5) and are thus binding on the Examiner.

2. "Notice of Non-Compliant Amendment (37 CFR 1.821 through 1.825" [sic, Non-Compliance with Sequence Disclosure Rules] (OA pp. 5-6).

2.1. As a preliminary matter, we note that the Examiner improperly refers to a "non-compliant amendment". A non-compliant amendment is one which fails to satisfy the formal requirements of 37 CFR 1.121. The Examiner has not pointed out any formal defect in the amendment filed May 25, 2005.

Rather, the Examiner is attempting to assert that the application is non-compliant with the sequence disclosure rules. We note that this is the first time the issue of compliance with the sequence disclosure rules has been raised in this case, so there was no prior formal requirement to submit a sequence listing. Hence, no prior response can be deemed non-responsive for failing to supply a sequence listing in response to a notice requiring such.

2.2. Applicants hereby submit the following:

a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification

as directed above; and
the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein.

2.3. The description has been amended to comply with §1.821(d).

2.4. The undersigned attorney or agent hereby states as follows:

(a) this submission does not include new matter [§1.821(g)];
(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are the same [§1.821(f) and §1.825(b)];
(c) if the paper copy has been amended, the amendment is supported by the specification and does not include new matter [§1.825(a)]; and
(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is identical to that originally filed [§1.825(d)].

2.5. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence

does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

3. Information Disclosure Statement (OA p. 6)

It was never contended that the discussion of references in the specification constituted an IDS. However, to the extent that these references have been made of record in the IDS of May 15, 2006, the discussion in the specification should be considered by the examiner as a voluntary comment on the relevance of the references.

4. Objections (OA pp. 6-8)

4.1. The drawing objection is moot since page 10, lines 15-21 has been amended to delete reference to Figure 1 and thus

there is no reason why Figure 1 should be amended to depict the enzymatic conversion(s) in question.

4.2. The specification objection is moot as the references to the non-existent Fig. 3 on pp. 11 and 17 have been deleted.

4.3. We submit that the amendments to the claims render the claims objection moot. However, we respectfully suggest that it not necessary or even helpful to spell out the standard biochemical terms NAD, NADP, NADH and NADPH and that the claims could be more clearly presented if the Examiner did not demand this. For instance, the reference to GAPN would probably be clearer if it read: 'NADP-dependent Glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.9 (GAPN)'

5. Definiteness Issus (OA pp. 8-9)

The Examiner has argued that the lack of a definition of the term 'native level' in respect of the third enzyme renders the term unclear as it might mean either:

a) the activity of the wild type enzyme with no amino acid substitutions; or

b) 'the functionality of a second enzyme in the presence of the cofactor in relation to the activity of the third enzyme'.

Neither was intended. Possibly the words 'in respect' have caused difficulty. An amendment has been made on that basis. What is intended is of course that there should be above a native level of activity of the third enzyme. In the illustrated case, the native activity of the third enzyme in the yeast is zero, because the native form of the yeast does not express GAPN. The wording is also apt to cover a situation in which the third enzyme activity is natively present, but has been increased such as by over expression.

Secondly, it has been argued that it is unclear whether the first metabolite is simultaneously transformed into a second metabolite and a third metabolite or whether these are

separate metabolic conversions. We in turn are confused as to what the Examiner means by 'simultaneously' and 'separately'.

The claim specifies that there should be transformation of the first metabolite into a second metabolite (which is in turn transformed into a further metabolite - that being clearly sequential to the 'first to second' transformation).

The claim further specifies that there should be transformation of the first metabolite into the third metabolite. Clearly these transformations of the first metabolite into the second and third metabolites are 'separate' because they start from the first metabolite and go to different products. Equally, they are expected to be 'simultaneous' in that both processes will be ongoing at the same time.

The dichotomy proposed by the Examiner does not seem to us to exist.

It has been further objected that the claims lack clarity because of the use of parentheses. However, we believe it to be standard practice to use parentheses to give an alternative name for a material, for instance where initials are followed by the full name of a compound or vice versa. The EC number provides the standard classification and thus helps to clarify which enzyme is intended for the enzyme in question. In many cases, the EC number refers to a single enzyme.

6. Prior Art Issues (OA pp. 9-12)

The Examiner has objected that the claimed subject matter (Claims 1-13) is obvious over Nissen et al in view of Valverde et al. We traverse.

Nissen et al is cited as disclosing a metabolically engineered *S. cerevisiae* wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield. To this end, a mutant *S. cerevisiae* was produced in which *GLN1* encoding glutamate synthetase, and *GLT1* encoding glutamate synthetase were over

expressed. *GDH1* encoding the NADPH-dependent glutamate dehydrogenase was deleted.

This led to consumption of 1 mol of NADH and ATP per mole of glutamate instead of 1 mol of NADPH, leading to a reduction of surplus formation of NADH, increased ethanol production and decreased glycerol production.

Nissen does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase (e.g. GAPN aka GAPDHN). Indeed, we would add that Nissen et al does not disclose reducing formation of NADH at all. When Nissen et al speaks of reduced formation of surplus NADH, this is not in fact via the mechanism of reduction of NADH formation, but via provision of a pathway for consuming it.

Valverde et al discloses a metabolically engineered *E. coli* in which the NAD-dependent glycolytic phosphorylating G3P dehydrogenase GAPDH was deleted and in which GAPN was expressed resulting in the reaction:

Glyceraldehyde 3-phosphate + NADP+ = 3-phosphoglycerate + NADPH.

As a result, the *E. coli* strain was unable to grow anaerobically on sugars but had recovered the ability to grow aerobically on sugars. It also failed to grow on gluconeogenic substrates (acetate + succinate) and showed a lower growth rate than wild type (col. 1, page 155).

The Examiner's contention is that because Nissen et al taught that reducing formation of NADH and increasing consumption of ATP resulted in decreased glycerol formation in yeast, it would have been obvious from Valverde et al that this effect could be obtained also by expressing GAPN in yeast to produce 3-phosphoglycerate with production of NADPH rather than NADH + ATP.

We submit that the rejection should be reconsidered and withdrawn.

First, Nissen et al's teaching is significantly misstated. It does not teach reduction in the production of NADH. Rather, it teaches provision of an NADH consuming reaction path that does not lead to glycerol. This undermines the Examiner's argument, which starts from a false premise.

Secondly, as explained in Nissen et al, one previous strategy for avoiding production of glycerol in yeast was to block the pathway leading to its production by deletion of genes encoding GPD1 and GPD2, but this led to a strain that could not grow under anaerobic conditions (col. 1, page 70). Nissen et al instead choose to drain off surplus formation of NADH by changing the cofactor requirement in amino acid synthesis.

One consequence of the metabolic engineering in Valverde et al is that the resulting *E. coli* is unable to grow anaerobically, the very problem that Valverde et al were trying to avoid. A skilled reader would therefore not perceive Valverde et al as offering a teaching likely to be useful in yeast as an alternative strategy for obtaining the objects of Nissen et al (reduced glycerol and increased ethanol).

Furthermore, it should be recognized that Valverde et al has nothing to say regarding the balance of production of any product by the metabolism of the engineered *E. coli*, let alone of glycerol/ethanol.

The two teachings lie in very different fields, one being concerned with the metabolism of a yeast and the other with the metabolism of a bacteria. A skilled person would be unlikely to look for an alternative solution to the problem addressed in Nissen et al relating to yeast in a teaching confined to the metabolism of *E. coli*.

It should further be recognized that the Examiner's rejection is dependent on the skilled worker choosing not to adopt the whole of the changes taught by Valverde et al and to apply them to yeast. Thus, the claims require that the yeast

should have an intact GAPDH which produces NADH, whereas in Valverde et al, the *E. coli* had its NAD-dependent GAPDH deleted. We submit that if it were obvious to combine the teachings of Nissen et al and Valverde et al at all (which we deny), the result would be a yeast in which, *per* Valverde et al, GAPDH would be deleted and GAPN would be introduced. That however would not meet the requirements of claim 1. The Examiner cannot properly pick and choose <u>which features</u> of Nissen and Valverde to combine on the basis of applicant's own teachings; that is hindsight reconstruction. Yet the Examiner fails to articulate any teaching in Nissen which would direct the ordinary worker to pick only the features of Valverde which the Examiner has emphasized.

Also, it should be noted that Valverde et al does not teach an alternative route by which one can obtain the same effect taught in Nissen et al. Whilst Nissen et al teaches a way to 'drain off' surplus production of NADH that will otherwise lead to glycerol production, no such draining off mechanism is taught or provided in Valverde et al. Accordingly, the proposed combination is not a substitution of like with like.

Instead, Valverde et al teaches that the ability to metabolize sugar lost via a deletion of GAPDH can be partially rescued (but only under aerobic conditions) by introducing GAPN. According to the Examiner, it is apparent that the catabolic yield of GAPN includes NADPH. This is clearly quite different from draining off NADH.

Even if the combination proposed by the Examiner were to be conceived by a skilled reader on the basis of combining Nissen et al and a part of the teaching of Valverde et al as the Examiner proposes, there would have been several reasons not to hold a reasonable expectation that the desired effect would be achieved.

First, it would have been unknown whether GAPN could be

expressed successfully and effectively in yeast.

Secondly, it would have been unknown whether production of NADPH via expression and activity of GAPN would have any substantial effect on the level of NADH in yeast. Here it should again be borne in mind that the expression of GAPN does not produce operation of the mechanism taught for reducing glycerol yield in Nissen et al. It does not operate to drain off NADH. Rather the hope on which the Examiner's argument depends would be that avoiding one route to the production of NADH by using GAPN to produce NADPH instead would have a material effect.

This would of course have been completely unknown. First, it would have been unknown to what extent GAPN if expressed successfully in yeast would become engaged in glycolysis when competing with native yeast enzymes. It should be borne in mind that Valverde et al had deleted the *E. coli* GAPDH so had not even demonstrated that GAPN would have a material effect in *E. coli* in which the native glycolysis pathway had not been destroyed, let alone that it would be effective in yeast.

Thirdly, as the Examiner has pointed out (page 12 of the action), a second consequence of metabolizing G3P to 3-PGA via GAPN rather than via GAPDH is that one does not get production of ATP. However, the effect on the production of glycerol and ethanol in a yeast of this loss of ATP production would have been quite unknown. As seen in the diagram on page 157 of Valverde et al, ATP is required for consumption in the earlier stages of metabolism of glucose. Valverde et al had reported that their engineered *E. coli* had a decreased growth rate compared to wild type. A skilled reader would have good grounds for expecting that the hypothesized transformed yeast would also have decreased growth rates.

We submit that a skilled reader would not have found it obvious to combine the teachings as proposed and would not have had a reasonable expectation that the combination would

achieve the desired end.

Respectfully submitted,

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